Department of Pharmacology and Toxicology
The Brody School of Medicine
East Carolina University
Room 6S10
600 Moye Blvd.
Greenville, NC 27834-4354

David A. Taylor, Ph.D.
Professor and Chairman
252-744-2734

Pharmacology Faculty
Abdel Abdel-Rahman, Ph.D.
Saeed Dar, Ph.D.
James Gibson, Ph.D.
Tatyana Ivanova-Nikolova, Ph.D.
Mona McConnaughey, Ph.D.
Brian McMillen, Ph.D.
Ken Soderstrom, Ph.D.
Rukiyah Van Dross, Ph.D.

Emeritus Faculty
Donald Barnes, Ph.D.
Alphonse Ingenito, Ph.D.
Robert Myers, Ph.D.
Wallace Wooles, Ph.D.

Website Address:
www.ecu.edu/pharmacology

Phone:
252-744-2734

Fax:
252-744-3203

December 1, 2006

Dr. David A. Taylor
Professor and Chair
Department of Pharmacology
Brody School of Medicine
East Carolina University

Dear Dr. Taylor:

During our conversation on November 29, 2006, in which you informed me about your decision and the decision of the Departmental Tenure and Promotion committee regarding my tenure and promotion, I inquired about the possibility of updating my PAD before the meeting of the BSOM Tenure and Promotion committee.

I was advised that the BSOM Tenure and Promotion committee finds it helpful to see the list of grant applications submitted to different granting agencies, together with the summary statements and scores when applicable. Therefore I would like to request your permission to add to my PAD the list of the grant applications to NIH and NSF, directly printed out from eCommons and FastLane together with the Panel Summaries (1 page each) for the three NSF grant proposals, and the AHA Applicant Notification for the latest AHA grant application. I am enclosing these documents for your review.

I will greatly appreciate your understanding of this matter.

Thank you very much for your help.

Respectfully,

Tatyana T. Ivanova-Nikolova, Ph. D.

Status Result - PI Status

Important: The NIH provides the JIT (Just in Time) link in the Commons for applications receiving a percentile of less than 30 or for applications receiving a priority score of between 100 and 300 if no percentile is provided. Please await instructions from the NIH on whether to complete this information. Furthermore, there is a system problem with the Commons, which shows the JIT link for NRSA applications (Fellowships and Training applications). Please do not submit the JIT information for these types of applications through the Commons. Please submit JIT information for training grants and fellowships through email or fax. Finally, JIT requires a Signing Official (SO) at your institution to send the request to the NIH. As a Principal Investigator, you are able to save this information. However, you must notify an individual with SO rights to forward the information to the NIH. Thank you for your cooperation.

PI Status: View Failed Prior eSubmissions

Total Result: 7

Application ID	Proposal Title	PI Name	Application Status	Status Date
1R01HL063369-01A1	Functional diversity of G protein-effector interactions	IVANOVA-NIKOLOVA, TATYANA T	Not Funded	10/13/2000
1R01HL079083-01	Vagal compliance of the heart as a therapeutic target	IVANOVA-NIKOLOVA, TATYANA T	Withdrawn	11/19/2004
1R01GM075230-01	Integration of G protein signal transduction	IVANOVA-NIKOLOVA, TATYANA T	Withdrawn	12/01/2005
1R01HL079083-01A1	Vagal compliance of the heart as a therapeutic target	IVANOVA-NIKOLOVA, TATYANA T	Not Funded	03/07/2005
1R01GM075230-01A1	Integration of G protein signal transduction	IVANOVA-NIKOLOVA, TATYANA T	Withdrawn	11/27/2006
1R21HL089728-01	Temporal dynamics of GIRK channel regulation in the heart	IVANOVA-NIKOLOVA, TATYANA T	Pending	10/23/2006
1R01GM075230-01A2	Intergration of G protein signal transduction	IVANOVA-NIKOLOVA, TATYANA T	Pending	11/27/2006

PI: IVANOVA-NIKOLOVA, TATYANA T	Title: Temporal dynamics of GIRK channel regulation in the heart	
Received: 10/03/2006	FOA: PA06-161	Council: 05/2007
	FOA Title: NIH EXPLORATORY/DEVELOPMENTAL RESEARCH GRANT PROGRAM (PARENT R21)	
1 R21 HL089728-01	Dual: AG	Accession Number: 2952337
IPF: 578209	Organization: EAST CAROLINA UNIVERSITY	
Former Number:	Department: Phamacology and Toxicology	
IRG/SRG: ESTA	AIDS: N	Expedited: N
Subtotal Direct Costs (excludes consortium F&A) Year 1: 125,000 Year 2: 125,000	Animals: Y Humans: N Clinical Trial: N Exemption: 10 HESC: N	New Investigator: N

Senior/Key Personnel:	*Organization:*	*Role Category:*
Tatyana Ivanova-Nikolova PhD	East Carolina University	PD/PI
Emil Nikolov	East Carolina University	Technician

16. ESTIMATED PROJECT FUNDING		17. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?
a. * Total Estimated Project Funding	$349,873.00	a. YES ○ THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON: DATE:
b. * Total Federal & Non-Federal Funds	$349,873.00	b. NO ● PROGRAM IS NOT COVERED BY E.O. 12372; OR
. * Estimated Program Income	$0.00	○ PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

18. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

● * I agree

** The list of certifications and assurances, or an internet site where you may obtain this list, is contained in the announcement or agency specific instructions.*

19. Authorized Representative

Prefix:	* First Name:	Middle Name:	* Last Name:	Suffix:
Ms.	Elizabeth	R	Eslick	

* Position/Title: Grants and Contracts Officer
* Organization Name: East Carolina University
Department: Office of Sponsored Programs
Division: Research and Graduate Studies
* Street1: 2200 South Charles Blvd
Street2: Greenville Centre, Rm 2906
* City: Greenville
County: Pitt
* State: NC: North Carolina
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 27858
* Phone Number: 252-744-1847
Fax Number: 252-328-4363
* Email: eslicke@ecu.edu

* Signature of Authorized Representative	* Date Signed
Elizabeth Eslick	10/03/2006

20. Pre-application File Name: Mime Type:

21. Attach an additional list of Project Congressional Districts if needed.

File Name: Mime Type:

APPLICATION FOR FEDERAL ASSISTANCE

SF 424 (R&R)

2. DATE SUBMITTED	Applicant Identifier
3. DATE RECEIVED BY STATE	State Application Identifier

1. * TYPE OF SUBMISSION

○ Pre-application ○ Application ● Changed/Corrected Application

4. Federal Identifier
56-6000403

5. APPLICANT INFORMATION * Organizational DUNS:607579018

* Legal Name: East Carolina University
Department: Office of Sponsored Programs
Division: Research and Graduate Studies
* Street1: 2200 South Charles Blvd
Street2: Greenville Centre, RM 2906
* City: Greenville
County: Pitt
* State: NC: North Carolina
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 27858

Person to be contacted on matters involving this application

Prefix:	* First Name:	Middle Name:	* Last Name:	Suffix:
Ms.	Elizabeth	R	Eslick	

* Phone Number: 252-744-1647
Fax Number: 252-328-4363
Email: eslicke@ecu.edu

6. * EMPLOYER IDENTIFICATION NUMBER *(EIN) or (TIN)*:
56-6000403

7. * TYPE OF APPLICANT
H: Public/State Controlled Institution of Higher Education

8. * TYPE OF APPLICATION: ● New
○ Resubmission ○ Renewal ○ Continuation ○ Revision

Other (Specify):
Small Business Organization Type
○ Women Owned ○ Socially and Economically Disadvantaged

If Revision, mark appropriate box(es).
○ A. Increase Award ○ B. Decrease Award ○ C. Increase Duration
○ D. Decrease Duration ○ E. Other *(specify)*:

9. * NAME OF FEDERAL AGENCY:
National Institutes of Health

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:
TITLE:

* Is this application being submitted to other agencies? ○ Yes ● No
What other Agencies?

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:
Temporal dynamics of GIRK channel regulation in the heart

12. * AREAS AFFECTED BY PROJECT *(cities, counties, states, etc.)*
Greenville, NC Pitt

13. PROPOSED PROJECT:

* Start Date	* Ending Date
07/01/2007	06/30/2009

14. CONGRESSIONAL DISTRICTS OF:

a. * Applicant	b. * Project
13	NC1&3

15. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION

Prefix:	* First Name:	Middle Name:	* Last Name:	Suffix:
	Tatyana	T	Ivanova-Nikolova	PhD

Position/Title: Assistant Professor
* Organization Name: East Carolina University
Department: Pharmacology and Toxicology
Division: Brody School of Medicine
* Street1: Brody School of Medicine, Rm 6S-10
Street2: 600 Moye Blvd
* City: Greenville
County: Pitt
* State: NC: North Carolina
Province:
* Country: USA: UNITED STATES
* ZIP / Postal Code: 27834
* Phone Number: 252-744-2757
Fax Number: 252-744-3203
* Email: ivanovanikolovat@ecu.edu

Tracking Number: | Funding Opportunity Number: | Received Date: Time Zone: GMT-5 | OMB Number: 4040-0001 Expiration Date: 04/30/2008

Abstract

G-protein-gated inwardly rectifying (GIRK) channels are key determinants of the inhibitory synaptic transmission in the heart. The canonical atrial GIRK channels are heterotetramers consisting of two homologous subunits, GIRK1 and GIRK4, and have unitary conductance of approximately 35 pS with symmetrical 150 mM KCl solutions. Recently we reported the characterization of a previously unknown small conductance GIRK (scGIRK) channel present in rat atria. The scGIRK channels are directly activated by Gβγ subunits and have a unitary conductance of 16 pS. Stimulation of m_2-muscarinic receptors in atrial myocytes activates both the GIRK1/4 and scGIRK channels. Localization of the two channels to the same membrane regions suggests that they might work in concert to generate a large repertoire of hyperpolarization responses. Here we will use a single-molecule analysis of channel function to test the hypothesis that differential modulation of GIRK1/4 and scGIRK channels by neurotransmitters plays an essential role in the integration of cholinergic and adrenergic pathways in the atria.

The knowledge gained from these studies would reveal new paradigms in the control of membrane excitability and might identify new pharmacological targets for treatment of cardiac dysfunction.

Research Plan

Specific Aims

The autonomic nervous system plays a fundamental role in the regulation of normal heart function. However, chronic changes in the equilibrium between the sympathetic and parasympathetic signals to the heart have detrimental effects on the cardiovascular system (1-4). A host of medical conditions, including chronic heart failure, diabetes and clinical depression, are associated with changes in the balance between sympathetic and parasympathetic signals to the heart. Yet, the molecular mechanisms that control this balance remain unknown. Therefore, it is critical to understand the complex interactions between the sympathetic and parasympathetic signals in cardiac myocytes and to develop therapeutic strategies aimed at restoring the normal equilibrium between these signals. G protein-regulated inwardly rectifying K^+ (GIRK or K_{ACh}) channels of the sinoatrial node and atria are key determinants of the inhibitory synaptic transmission in the heart. These channels translate the parasympathetic signals mediated through m_2-muscarinic receptor (M2R) into membrane hyperpolarization and are responsible for the ensuing reduction of the heart rate (5-7). Recently, we established that in the atrial membrane the canonical 35-pS GIRK1/4 channels coexist with previously unknown 16-pS GIRK (scGIRK) channels (8). More importantly, the scGIRK channels are capable of undergoing a transformation from Gβγ scavengers, in the presence of Gβγ alone, to highly efficient partners of traditional GIRK1/4 channels, after application of PKA. The ability of scGIRK channels to switch their function suggests that this novel type of GIRK channels might function together with GIRK1/4 channels as coordinators between the sympathetic and parasympathetic signals in the atria.

This proposal will test the hypothesis that dynamic regulation of GIRK1/4 and scGIRK channels by neurotransmitters plays an essential role in the integration of cholinergic and adrenergic pathways in the atria. Initially, we will focus on the integration of signals transmitted through M2 and α-adrenergic receptors (AR). This combination provides an optimal venue for testing our hypothesis because of the well established ability of different G_q-coupled receptors to inhibit GIRK1/4 channel currents (reviewed in Ref. 9). Furthermore, recent studies from our laboratory revealed the dynamic nature of such inhibition. Thus, we established that stimulation of M2Rs alone generates a sustained GIRK1/4 activation, whereas concurrent stimulation of M2 and α-adrenergic receptors induces transient GIRK1/4 channel activation. Here we will determine whether scGIRK channel activity is also altered in the process of integration of M2- and α-ARs signals. In addition, since the G_q-coupled receptors recruit a large repertoire of parallel cellular processes to accomplish the inhibition of GIRK1/4 channel currents, we will also focus on individual factors producing this inhibition. Depletion of phosphatidylinositol-4, 5-bisphosphate (PIP_2), activation of protein kinase C (PKC), and direct binding of $G\alpha_q$ subunits to the channel have been all shown to contribute to this phenomenon (9-16). How these processes modulate GIRK1/4 channel function at single-molecule level remains less well understood. Therefore, we will delineate the individual contributions of PIP_2 depletion and PKC activation to the regulation of both the GIRK1/4 and scGIRK channels, using single-molecule electrophysiological analysis.

While it is well established that activation of atrial GIRK channels results from direct binding of G protein βγ subunits, the single-channel recordings reveal dynamic GIRK molecules, fluctuating between several patterns of activity (8, 17). Therefore, we have previously developed a methodology for classification of heterogeneous GIRK channel behavior into different functional modes and for detection modal transitions in channel gating. This methodology has enabled us to monitor the dynamic interactions between Gβγ and GIRK channels in real time and to build a conceptual framework for interpretation of these interactions (8). Here we will combine this biophysical methodology with biochemical methods to achieve the following specific aims:

Specific aim 1: To establish the temporal dynamics of scGIRK channel regulation by M2- and α-ARs in the atria.

Specific aim 2: To delineate the individual contribution of PIP_2 and PKC to the dynamic modulation of atrial GIRK channels by α-ARs.

Accomplishing these goals will lead to a better understanding of the fundamental mechanisms underlying the coordination of sympathetic and parasympathetic signals in atrial myocytes. Furthermore, it will

be essential for the design of novel AR-targeting therapies aimed at achieving an optimal autonomic control of heart function in numerous clinical conditions.

Proposal Status | MAIN ▸

Organization: East Carolina University

Search for Proposals by any of the following:

Proposal Number: (Enter 7 digits)

Received By NSF Date Range (*mm/dd/yyyy*)
From: To:

Search

All Proposals by Status:

To view detailed proposal status information, click the "Proposal Number" (e.g.: 0003337) link below. Sort results by clicking column titles.

Principal Investigator's Name: Tatyana Ivanova-Nikolova

4 proposals found, displaying 1 to 4 1

Division	Proposal Number	Proposal Title	Performing Organization	Status	Status Date	Received by NSF	Requested Amount	Program Officer
EF	0652608	FIBR Preliminary Proposal: How do cells integrate opposing signals?	East Carolina University	Pending	09/28/2006	09/22/2006	$2.00	Parag R. Chitnis
MCB	0615599	Coordination of G protein signaling	East Carolina University	Declined	06/12/2006	01/12/2006	$492,601.00	N/A
MCB	0517485	Coordination of G protein signaling	East Carolina University	Declined	07/06/2005	01/12/2005	$492,600.00	N/A
MCB	0416991	Coordination of G protein signaling	East Carolina University	Declined	06/21/2004	01/12/2004	$578,794.00	N/A

Export options: Excel

Download Adobe Acrobat Reader for viewing PDF files

National Science Foundation
4201 Wilson Boulevard, Arlington, Virginia 22230, USA
Tel: 703-292-5111, FIRS: 800-877-8339 | TDD: 703-292-5090

Proposal Status | MAIN ▸

Organization: East Carolina University

Proposal Detail:

Proposal Information

Proposal Number:	0652608
Proposal Title:	FIBR Preliminary Proposal: How do cells integrate opposing signals?
Received by NSF:	09/22/06
Principal Investigator:	Tatyana Ivanova-Nikolova
Performing Organization:	East Carolina University

This Proposal has been Electronically Signed by the Authorized Organizational Representative (AOR).

NSF Program Information

NSF Division:	Emerging Frontiers
NSF Program:	Frontiers in Biological Research (FIBR)
Program Officer:	Parag R. Chitnis
PO Telephone:	(703) 292-8444
PO Email:	pchitnis@nsf.gov
Proposal Classification Form:	Submitted

> View/Print Proposal Classification Form

Review Information: External Peer Review began on **11/29/06**

Proposal Status

Status As of Today Dated: **12/01/06**

This preliminary proposal is currently being considered by the above Program. Principal Investigator will receive notice of the outcome of the preliminary proposal once the review process has been completed.

◂ Back to Status Search Results

Download Adobe Acrobat Reader for viewing PDF files

Proposal Status | MAIN

Organization: East Carolina University

Panel Summary #1

Proposal Number: 0615599

Panel Summary:
Panel Summary

Signal Transduction and Cellular Regulation Panel

Review Criterion I: Intellectual Merit (Strengths and Weaknesses)

The main goal of this proposal is to understand the regulation of GIRK channels. The PI has good preliminary results which is major strength of this application. The PI submitted an upadate that demonstrated further progress.

However, there are problems particularly concerning the second specific aim. First, the study focuses only on known proteins and may not reveal new proteins. Second, the antibody may bring down other non-specific proteins. In addition, it was not clear if the technique would have enough sensitivity to probe dynamics (Specific aim 2B).

The panel felt that the review that raised the issue of publication record is unduely harsh.

Review Criterion II: Broader Impact (Strengths and Weaknesses)

This proposal employs advanced techniques. There was some concern whether high school students may be able to handle these experiments.

Summary Comments:

This is a good proposal with good preliminary results. However, potential problems concerning the analysis of GIRK signaling complexes reduced enthusiasms of the panel.

Note: The summary was read by/to the panel and the panel concurred that the summary accurately reflects the panel discussion.

Panel Recommendation: Medium

Back to Proposal Status Detail

Proposal Status | MAIN

Organization: East Carolina University

Panel Summary #1

Proposal Number: 0517485

Panel Summary:
Signal Transduction and Cellular Regulation Panel

Positive And Unique Aspects of the Proposal:
The PI will test the hypothesis that formation of a physiologically relevant signaling network coordinates cholinergic and adrenergic signaling in myocytes.

Intellectual Merit (Review Criterion 1):
The PI has identified an antibody, 4A9, directed against the phosphorylated GIRK channel that was used to IP GIRK and associated proteins. Interestingly, in addition to the beta-gamma subunits of GPCRs, adrenergic effectors such as PKA, PKC, PP1 and PP2A were found to be associated with the GIRK channel. This is an intriguing finding as it suggests that GIRK channels might coordinate G-protein-coupled signaling pathways that are critical for membrane excitability and cell function. To investigate this conclusion the PI proposes a combination of electrophysiological and biochemical approaches.

Broader Impact (Review Criterion 2):
The educational activities of the PI are very good. The proposed research will include students at all levels including high school students.

Summary Comments:
This is a high-risk high-reward proposal. The PI is well qualified to perform the electrophysiological component of the proposal. However, the panel felt that the biochemical component needs improvement, and that the aims should be simplified. Furthermore, the panel agreed that the PI needs to show a functional effect on channel gating induced by adrenergic agonists as preliminary results before funding can be initiated.

Note: The summary was read by/to the panel and the panel concurred that the summary accurately reflects the panel discussion.

Panel Recommendation: Recommended

Back to Proposal Status Detail

National Science Foundation
4201 Wilson Boulevard, Arlington, Virginia 22230, USA
Tel: 703-292-5111, FIRS: 800-877-8339 | TDD: 703-292-5090

Proposal Status | MAIN

Organization: East Carolina University

Panel Summary #1

Proposal Number: 0416991

Panel Summary:
Signal Transduction Spring Panel
April 19-21, 2004

Positive and Unique Aspects of the proposal

The applicant has preliminary data suggesting the existence of ion-channel associated complexes containing signalling molecules associated with the channel. This is a novel and exciting concept.

Criterion I: Intellectual Merit (Strengths and Weaknesses)

The results on the existence of the ion-channel associated signalling complex based upon immunoprecipitation with a monoclonal antibody to phoshoserine are novel and exciting. However, the panel questioned the specificity of the antibody and felt that further characterization of the antibody and the multiprotein complex is needed. In addition, there was a concern that the presence of actin in the associated complex was most probably artifactual.

Criterion II: Broader Impact (Strengths and Weaknesses)
If verified, the scientific hypothesis has far reaching implications for the function of ion-channels. Although ECU has programs for undergraduate education involving minorities, the applicant does not consider broader participation in educational opportunities.

Synthesis
This is a potentially exciting and innovative proposal from a beginning investigator that would be improved by more detailed consideration of the specificity of the antibody used and by including a plan for broader impact of the research.

Overall Rating:MERITORIOUS

The summary was read by/to the panel and the panel concurred that the summary accurately reflects the panel discussion.

Panel Recommendation: Meritorious

Back to Proposal Status Detail

Ivanova-Nikolova, Tatyana

From: AHA Research Administration [awards@heart.org]
Sent: Tuesday, May 23, 2006 10:12 AM
To: Ivanova-Nikolova, Tatyana
Subject: AHA Applicant Notification - Reference Number: 0655469U

AHA Reference Number: 0655469U

Dear Tatyana Ivanova-Nikolova:

The American Heart Association (AHA) Mid-Atlantic Affiliate has completed the peer review process for your application. Your average priority score and percentile rank are listed below. If your application received a percentile rank between 0.01 and 49.99 percent, you will be notified of your status by email within two weeks. If your application received a percentile rank of 50 or greater, it cannot be funded (by AHA policy), and this message will serve as your final notification.

Your average priority score: 2.3
Your percentile rank: 45.35

The priority scores are based on a scale of one to five (a score of 1.0 - 1.4 being considered 'excellent'). The percentile rank from each peer review committee is based on a 0.01% to 99.99% ranking, with the most meritorious ranked application corresponding to the lowest percentile rank. The percentile rank is the relative rank of an application among those evaluated by a specific peer review committee.

Reviewer critiques are attached to this message. Please note that discussion comments may not always be reflected in the critique, due to its preparation in advance of the committee meeting. The AHA does not have a formal appeals process. Unsuccessful applicants are encouraged to reapply, if eligible for the program. Please save the application identification number for future resubmissions. Questions regarding critiques may be directed to peerreview@heart.org.

Please visit our Web site at www.myamericanheart.org/portal/professional/research for information regarding National Center application deadlines in July 2006. Visit the site after September 15th for information on the Mid-Atlantic Affiliate deadline in January 2007. Application inquiries may be directed to lora.wong@heart.org.

This email contains all of the information specific to the review of your application that we are able to share at this time. Thank you for submitting your application to the American Heart Association.

American Heart Association
Research Administration

------- Reviewer #1's Critique -------

A. 0655469U Tatyana Ivanova-Niklova Functional role of a novel GIRK channel in atrial myocytes

B. This is a revised application.
The objective is to characterize the effects of á and two kinds of â adrenergic signaling on small-conductance, G-protein-coupled inward rectifying K channels (scGIRK) in atria.

The major criticisms previously were the very small time commitment of the PI, the issue of interaction between scGIRK and the canonical GIRK channels in atria, and the scope of the project. All of these criticisms have been adequately addressed. A major improvement is the emphasis of recording from cell-attached patches in the protocol, allowing preservation of the intracellular signaling components for at least some of the time. New preliminary results uphold this idea, and other new results show changes in multimerization stoichiometry after adrenergic stimulation.

The research design and methods are to use the PI's previously developed and complex analytical scheme of G-protein ãã

subunits to these channels, and to dissect the effect of norepinephrine into its á1, â1 and â2 components using selective blockers. The analysis will yield a picture of sensitivity of the channel to beta/gamma subunits. The basic underlying theme is that there are 4 binding sites on the channel, and binding of 0 to 4 subunits leads to distinctive gating modes. The fundamental earlier finding is that these modes fit well with a binomial distribution with the exception of an excess of 0 binding.

C. Critique
This is an excellent application. The research topic is clearly important. The PI has published 2 recent and excellent papers on the topic, and several earlier excellent papers on related topics. In addition to high-quality electrophysiology, there are high-quality statistical methods.

1. Scientific excellence of the proposed research plan.

The Background section is very clear and informative. The Preliminary Results recapitulate her 2 papers from 2004.

Aim 1 is to establish the role of á AR signaling in the regulation of scGIRK channels in atrial myocytes. The method is to add phenylephrine (now pre-added prior to cell-attached seal formation) and propranolol.

Aim 2 is to delineate the contribution of â1 and â2 ARs to the regulation of scgIRK channels. She will dissect contributions of â1 and â2 adrenergic receptors using norepinephrine with combinations of specific adrenergic blockers.

2. Investigator: qualifications of the applicant

The qualifications of the investigator are excellent. She did post-doctoral work with G Breitweiser at Hopkins and J Robishaw at Geisinger, all on the theme of muscarinic K channels in atrium. These are outstanding investigators, and her published work from those experiences is exemplary.

The productivity of the investigator is good. She has published detailed and complete papers in JGP (1993, 1997 and 1998), and in both JBC and Biophysical Journal in 2004. The number of papers is not very large, but they are excellent and obviously she is the major player in them.

The independence of the investigator is unquestioned.

3. Quality of the facilities and resources available from the institution are certainly adequate.

The available facilities are a patch clamp setup and cell culture facilities, and equipment for biochemistry experiments.

-------- Reviewer #1's Addendum --------

-------- Reviewer #2's Critique --------

A. Tatyana Ivanova-Nikolova
Functional role of a novel GIRK channel in atrial myocytes

B. Proposal Description:
G protein-regulated inward rectifying potassium (GIRK) channels are present throughout the body where they regulate the cell membrane resting potential. In cardiac atrial and sinoatrial nodal cells GIRK channels consist of a heterotetrameric arrangement of GIRK1 and GIRK4 subunits. Activation of the GIRK channels via muscarinic M2 receptors is directly responsible for the maintenance of vagal tone in the heart. The investigator has recently identified a small conductance GIRK (scGIRK) channel in atrial myocytes that exists along with the larger conductance "canonical" GIRK1/4 channel. This proposal will test the hypothesis that stimulation of adrenergic receptors promotes changes in the activity of scGIRK channels and their sensitivity to muscarinic stimulation. There are two aims of this project. In the first aim the PI will examine the role of alpha-adrenergic stimulation on the scGIRK channel. In the second aim the effect of beta1 and beta2-

adrenergic stimulation will be determined.

C. Critique

1. Scientific Significance and Approach
In cardiac atrial cells a "canonical" 35 pS GIRK channel is activated during acetylcholine stimulation of muscarinic M2 receptors. This GIRK or KACh channel is activated through the beta/gamma subunits of a pertusis-toxin sensitive G protein. In addition the KACh channel can be modulated by free fatty acids and metabolites of arachidonic acid. Of particular relevance to the present application, this channel is also regulated by both protein kinase-mediated phosphorylation and protein phosphatase-mediated dephosphorylation. In addition to the larger conductance 35 pS GIRK channel, a smaller 16 pS GIRK channel (scGIRK) was identified in neonatal rat atrial myocytes by the investigator's laboratory. The preliminary results shown in Figures 8-10 demonstrate that the catalytic subunit of protein kinase A (PKA) and the alpha-adrenergic agonist phenylephrine regulate scGIRK channels. These experiments are the basis for the interesting hypothesis that adrenergic receptor stimulation may have differing actions on the large and small conductance GIRK channels. This could then allow for some type of integrated action of muscarinic and adrenergic receptor signaling effects on the heart. The major strengths of the proposal include the strong preliminary results, the importance of the area under study and the experience of the investigator in single channel acquisition and analysis. The chief weakness of the proposal is that the experimental design does not extend beyond the simple paradigm of treating myocytes with adrenergic agents and then recording single channel activity. As pointed out in a previous review, this gives the overall impression of a project that is limited in scope.

This grant proposal is a revised application. The major scientific criticisms of the previous proposal included 1) the "interactions" between the two conductance channels in the original Aim #1 were not well-defined (Revs 1 and 2), 2) the number of experiments was limited for a two year study (Rev 1) and 3) the excision of membrane patches may cause the diffusion of adrenergic receptor signaling molecules (Rev 2). The investigator has responded to the first criticism by eliminating studies of adrenergic effects on the interactions of the two channels. This appears to actually decrease the number of proposed experiments (contrary to criticism #2). The investigator indicates that obtaining high quality single channel recordings is time consuming and limits the number of experiments. In response to criticism #3, the investigator has included new data showing that alpha-adrenergic stimulation regulates the GIRK channels in cell attached patches. This data, along with the PKA experiments, provide convincing evidence that adrenergic pathways may have differing actions on the two channels. However, since cell-attached recordings become unstable with time, the investigator still plans to carry out excised patch experiments with the beta/gamma G protein subunits. It seems likely that if endogenous protein kinases/phosphatases become activated during beta or alpha-adrenergic stimulation, that these signaling molecules would diffuse out of the membrane patch. It is unclear why the investigator has not proposed studies similar to those displayed in figure 8 with PKA. Since the downstream signaling molecules activated by cardiac beta and alpha-adrenergic receptors have been identified, application of these molecules (along with the G protein subunits) would extend the experimental design. In addition, these experiments would address the previous criticisms. Thus, although the proposal has been improved through the inclusion of new data, weaknesses pointed out in the previous and current review remain.

2. Investigator
Dr. Ivanova-Nikolova is currently an Assistant Professor in the department of Pharmacology and Toxicology at the East Carolina University, School of Medicine. Dr. Ivanova-Nikolova earned her Ph.D. from the Institute of Biological Physics at the USSR Academy of Sciences. In addition to postdoctoral positions at LSU and Johns Hopkins University, the investigator has trained with Dr. Greta Breitwieser. The investigator recently published two high quality papers on the KACh channel from her laboratory at East Carolina. The investigator is well qualified to carry out the project.

3. Resources & Environment
The research environment and facilities are appropriate for the proposed studies.

------- Reviewer #2's Addendum -------